Claims

1. A method for determination of the concentration of free unbound hydrophobic Coenzyme A ester in a sample comprising the steps of
   - i) providing a hydrophobic Coenzyme A binding construct exhibiting a first signal when unbound and exhibiting a measurably different second signal when bound to a hydrophobic Coenzyme A ester,
   - ii) contacting the sample with the labelled hydrophobic Coenzyme A binding construct,
   - iii) allowing at least one species of unbound free hydrophobic Coenzyme A ester to bind to the hydrophobic Coenzyme A binding construct forming a complex comprising a hydrophobic Coenzyme A ester and the hydrophobic Coenzyme A binding construct,
   - iv) detecting a signal from the complex,
   - v) correlating the signal to the concentration of the at least one species of hydrophobic Coenzyme A ester in the sample.

2. The method according to claim 1, wherein the hydrophobic Coenzyme A binding construct comprises a heterologous peptide capable of binding at least one species of hydrophobic Coenzyme A ester and a signal moiety.

3. The method according to claim 2, whereby heterologous peptide comprises an acyl-CoenzymeA binding protein, a variant or functional equivalent thereof.

4. The method according to claim 3, whereby the acyl-Coenzyme A binding protein comprises an amino acid sequence from the sequences of Figure 1 (SEQ ID NO 1 to 30) a variant or functional equivalent thereof.

5. The method according to claim 2, whereby the heterologous peptide comprises bovine ACBP, a variant or functional equivalent thereof.

6. The method according to claim 2, whereby the heterologous peptide comprises a cystein or lysin residue for binding the signal moiety.

7. The method according to claim 6, whereby one native amino acid residue in the heterologous peptide has been substituted by a cystein or a lysin residue for binding the signal moiety.

8. The method according to claim 6, whereby the residue is selected from the amino acid residues aligning an acyl Coenzyme A binding domain.

9. The method according to claim 6, whereby the residue is selected from the amino acid residues having van der Waals' contact with a bound hydrophobic Coenzyme A ester.

10. The method according to claim 6, whereby the residue is selected from the amino acid residues being within 5 Å from a bound hydrophobic Coenzyme A ester.

11. The method according to claim 6, whereby the residue is selected from the amino acid residues making up the α-helices of the heterologous peptide.

12. The method according to claim 6, whereby the heterologous peptide comprises the bovine ACBP and the native amino acid being replaced by a cystein residue is selected from the group consisting of Met-24, Leu-25, Ala-53, Asp-21, Lys-50, Lys-54, Lys-18, pro-19, Ala-9, Tyr-31, Lys-32, Tyr-28, Tyr-73, Val-12, Lys-13, Leu-15; Ile-27; more preferably whereby the native amino acid is selected from the group consisting of Met-24, Ala-53, and Lys-50.

13. The method according to claim 1, whereby the complex formed during step iii) has a K_D below 2 μM, such as below 1.5 μM, for example below 1.0 μM, preferably below 500 nM, more preferably below 200 nM such as below 100 nM, for example below 90 nM, such as below 80 nM, for example below 70 nM, such as below 60 nM, for example below 50 nM, such as below 40 nM, for example below 30 nM, such as below 20 nM, for example below 15 nM, such as below 10 nM, for example below 8 nM, such as below 7 nM, for example below 6 nM, such as below 5 nM, for example below 4 nM, such as below 3 nM, for example below 2 nM, such as below 1 nM, for example below 0.5 nM, such as below 0.1 nM.

14. The method according to claim 13, whereby the complex formed during step iii) has a higher K_D with respect to other species of hydrophobic Coenzyme A esters.

15. The method according to claim 14, whereby the one species of hydrophobic Coenzyme A ester is selected from the group consisting of acyl Coenzyme A esters having a C2 acyl group, a C4 acyl group, a C6 acyl group, a C8 acyl group, a C10 acyl group, a C12 acyl group, a C14 acyl group, a C16 acyl group, a C18 acyl group, a C20 acyl group, a C22 acyl group, a C24 acyl group, a C26 acyl group, a saturated acyl group, a mono-unsaturated acyl group, a polyunsaturated acyl group, an acyl group comprising a cis double bond, an acyl group comprising a trans double bond, an acyl group comprising a ring structure, an acyl group comprising a side chain.

16. The method according to claim 1, whereby the signal comprises a fluorescence signal.

17. The method according to claim 1, whereby the detected signal is essentially proportional to the amount of hydrophobic Coenzyme A ester in the sample.

18. The method according to claim 17, whereby the detected signal is essentially proportional to the amount of at least one species of Coenzyme A ester in the sample.

19. The method according to claim 18, whereby the at least one species of Coenzyme A ester comprises a species selected from the group consisting of Coenzyme A esters with a C2 acyl group, a C4 acyl group, a C6 acyl group, a C8 acyl group a C10 acyl group, a C12 acyl group, a C14 acyl group, a C16 acyl group, a C18 acyl group, a C20 acyl group, a C22 acyl group, a C24 acyl group, a C26 acyl group, a saturated acyl group, a mono-unsaturated acyl group, a polyunsaturated acyl group, an acyl group comprising a cis double bond, an acyl group comprising a trans double bond, an acyl group comprising a ring structure, an acyl group comprising a side chain.

20. The method according to claim 1, whereby the detected signal from a first species of hydrophobic Coenzyme A ester is essentially 0 and the detected signal from a second species of hydrophobic Coenzyme A is essentially proportional to the amount of said second species in the sample.

21. The method according to claim 1, whereby the detected signal is essentially proportional to the amount of a group of hydrophobic Coenzyme A esters in the sample.

22. The method according to claim 1, further comprising a step prior to step ii) wherein hydrophobic acids in the sample are converted to hydrophobic Coenzyme A esters by acyl Coenzyme A ligase.

23. The method according to claim 21, further comprising a prior step wherein triacylglycerides in the sample are converted to glycerol and free fatty acids.

24. The method according to claim 21, further comprising a prior step wherein phospholipids in the sample are converted to glycerol and free fatty acids.

25. The method according to claim 1, whereby the sample is selected from the group consisting of blood, urine, milk, tears, faeces, sperm, cerebrospinal fluid, nasal secrete, food, feed and mixtures, dilutions, or extracts thereof.

26. A construct for binding hydrophobic Coenzyme A ester comprising

i) a heterologous peptide capable of binding at least one species of hydrophobic Coenzyme A ester,

ii) a signal moiety.

27. The construct according to claim 26, wherein the signal moiety comprises a fluorescent moiety.

28. The construct according to claim 26, wherein the signal moiety exhibits a first signal when the construct is unbound and a measurably different second signal when the construct is bound to a hydrophobic-Conenzyme A ester.

29. The construct according to 26, wherein the signal moiety comprises (6-bromoacetyl-2-dimethylaminonaphtalene) BADAN.

30. The construct according to claim 27, wherein the fluorescent moiety comprises a compound selected from the group consisting of acrylodan; 5-dimethylaminonaphtalene-1-sulfonyl aziridine (danzyl aziridine); 4-[N-[2-iodoacetoxy)ethyl]-N-methylamino]-7-nitrobenz-2-oxa 1,3 diazole ester (IANBDE); 4-[N-[2-iodoacetoxy)ethyl]-N-methylamino]-7-nitrobenz-2-oxa 1,3 diazole amide (IANBDA); 6-acryloyl-2-dimtheylaminonaphtalene (acrylodan); N-(7-chlorobenz-2-oxa-1,3-diazyl-4-yl)sulfonyl morpholine; 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD chloride); didansyl-L-cystine; N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD amide); 7-fluorobenz-2-oxa-1,3-diazole-4-sulfonamide (ABD-F); 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD fluoride); 2-(4'-(iodoacetamido)anilino)naphtalene-6-sulfonic acid, sodium salt (IAANS); 5-(((2-iodoacetyl)amino)ethyl)amino)naphtalene-1-sulfonic acid (1,5-IAEDANS); 2-(4'-maleimidylanilino)naphtalene-6-sulfonic acid (MIANS); N-(1-pyreneethyl)iodoacetamide; N-(1-pyrene)iodoacetamide; N-(1-pyrene)maleimide; N-(1-pyrenemethyl)iodoacetamide (PMIA amide); 1-pyrenemethyl iodoacetate (PMIA ester); N-(1-pyrenepropyl)iodoacetamide); 1-(2,3-epoxypropyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium trifluoromethanesulfonate (PyMPO epoxide); erythrosin-5-iodoacetamide; fluorescein-5-maleimide; 5-iodoacetamidofluorescein (5-IAF); 6-

iodoacetamidofluorescein (6-IAF); 1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl)pyridinium methanesulfonate (PyMPO maleimide); Oregon Green™ 488 iodoacetamide "mixed isomers"; tetramethylrhodamine-5-iodoacetamide (5-TMRIA) "single isomer"; tetramethylrhodamine-5-maleimide "single isomer"; tetramethylrhodamine-6-maleimide "single isomer"; Texas Red® C_5 bromoacetamide; Texas Red® C_2 maleimide;

31. The construct according to claim 26, further comprising a second signal moiety.

32. The construct according to claim 31, wherein the second signal moiety is selected from the group of claim 30.

33. The construct according to claim 26, wherein the heterologous peptide comprises an acyl-CoenzymeA binding protein, a variant or functional equivalent thereof.

34. The construct according to claim 33, wherein the acyl-Coenzyme A binding protein comprises an amino acid sequence from the sequences of Figure 1 (SEQ ID NO 1 to 30) a variant or functional equivalent thereof.

35. The construct according to claim 26, wherein the heterologous peptide comprises an acyl-CoenzymeA binding domain.

36. The construct according to claim 26, wherein the heterologous peptide comprises bovine ACBP, a variant or functional equivalent thereof.

37. The construct according to claim 26, wherein the signal moiety is bound to a cystein or a lysin residue comprised in the heterologous peptide.

38. The construct according to claim 37, wherein the residue is non-native to the peptide.

39. The construct according to claim 37, wherein the residue is selected from the amino acid residues aligning an acyl Coenzyme A binding domain.

40. The construct according to claim 37, wherein the residue is selected from the amino acid residues being within 5 Å from a bound hydrophobic Coenzyme A ester.

41. The construct according to claim 37, wherein the residue is selected from the amino acid residues making up α-helices of the heterologous peptide.

42. The construct according to the claim 38, wherein the heterologous peptide comprises the bovine ACBP and the native amino acid being replaced by a cystein residue is selected from the group consisting of Met-24, Leu-25, Ala-53, Asp-21, Lys-50, Lys-54, Lys-18, pro-19, Ala-9, Tyr-31, Lys-32, Tyr-28, Tyr-73, Val-12, Lys-13, Leu-15; Ile-27; more preferably wherein the native amino acid is selected from the group consisting of Met-24, Ala-53, and Lys-50.

43. The construct according to claim 26, having a K_D with respect to at least one hydrophobic Coenzyme A ester below 2 μM, such as below 1.5 μM, for example below 1.0 μM, preferably below 500 nM, more preferably below 200 nM such as below 100 nM, for example below 90 nM, such as below 80 nM, for example below 70 nM, such as below 60 nM, for example below 50 nM, such as below 40 nM, for example below 30 nM, such as below 20 nM, for example below 15 nM, such as below 10 nM, for example below 8 nM, such as below 7 nM, for example below 6 nM, such as below 5 nM, for example below 4 nM, such as below 3 nM, for example below 2 nM, such as below 1 nM, for example below 0.5 nM, such as below 0.1 nM.

44. The construct according to claim 43, having a K_D with respect to one species of hydrophobic Coenzyme A ester below 2 μM, such as below 1.5 μM, for example below 1.0 μM, preferably below 500 nM, more preferably below 200 nM, such as below 100 nM, for example below 90 nM, such as below 80 nM, for example below 70 nM, such as below 60 nM, for example below 50 nM, such as below 40 nM, for example below 30 nM, such as below 20 nM, for example below 15 nM, such as below 10 nM, for example below 8 nM, such as below 7 nM, for example below 6 nM, such as below 5 nM, for example below 4 nM, such as below 3 nM, for example below 2 nM, such as below 1 nM, for example below

0.5 nM, such as below 0.1 nM and a higher K_D with respect to other species of hydrophobic Coenzyme A esters.

45. The construct according to claim 44, wherein the one species of hydrophobic Coenzyme A ester is selected from the group consisting of acyl Coenzyme A esters having a C2 acyl group, a C4 acyl group, a C6 acyl group, a C8 acyl group, a C10 acyl group, a C12 acyl group, a C14 acyl group, a C16 acyl group, a C18 acyl group, a C20 acyl group, a C22 acyl group, a C24 acyl group, a C26 acyl group, a saturated acyl group, a mono-unsaturated acyl group, a polyunsaturated acyl group, an acyl group comprising a cis double bond, an acyl group comprising a trans double bond, an acyl group comprising a ring structure, an acyl group comprising a side chain.

46. A kit for detection of the concentration of a hydrophobic Coenzyme A ester in a sample comprising

    i) at least a first construct according to claims 26 to 45,

    ii) a sample compartment for application of the sample.

47. The kit according to claim 46, further comprising an acyl-Coenzyme A synthetase, coenzyme A, adenosinetriphosphate, Mg^{++}, an antioxidant, and buffer.

48. The kit according to claim 47, further comprising pyrophosphatase.

49. The kit according to claim 47, further comprising a lipase, and buffer.

50. The kit according to claim 47, further comprising a phospholipase such as phospholipase A1 and/or A2, and buffer.

51. The kit according to claim 46, further comprising albumin.

52. The kit according to claim 46, wherein compounds are freeze dried.

53. The kit according to claim 46, wherein the hydrophobic-Coenzyme A ester binding construct is immobilised.

54. The kit according to claim 53, wherein the construct is immobilised in at least two different places, such as at least 3, for example at least 4 such as at least 5 different spaces.

55. The kit according to claim 46, comprising a second hydrophobic-Coenzyme A ester binding construct according to claims 26 to 45.

56. The kit according to claim 55, further comprising at least a third construct, such as at least a third and a fourth construct, for example at least a third, a fourth and a fifth construct.

57. The kit according to claim 55 or 56, wherein each construct has a K_D with respect to at least one species or a group of species of hydrophobic Coenzyme A esters, which is substantially lower than the K_D of the other construct(s) with respect to this species or group of species.

58. The kit according to claim 57, wherein substantially lower is 10 times lower, preferably 100 times lower.

59. The kit according to claim 55, wherein the first construct is a fluorescence acyl-CoA sensor 1 (FACI 24) and the second construct is a fluorescence acyl-CoA sensor 2 (FACI 53).

60. A method for determining the amount of free hydrophobic carboxylic acid(s) and/or lipid constituents in a sample comprising
   i. optionally fractionating the sample to obtain a substantially cell-free sample,
   ii. mixing the substantially cell-free sample with an amount of water-miscible organic solvent to precipitate proteins and obtain a solution of free fatty acids,
   iii. subjecting a sample of the supernatant to a quantitative analysis determining the amount of free fatty acids in the sample.

61. The method according to claim 60, wherein the sample comprises a blood sample and the substantially cell-free sample is serum.

62. The method according to claim 60, wherein the alcohol comprises a low molecular weight alcohol selected from the group consisting of ethanol, methanol, 1-propanol, 2-propanol, cyclopropanol.

63. The method according to claim 60, whereby the low molecular weight alcohol is selected from the group consisting of ethanol and 1-propanol.

64. The method according to claim 60, whereby the low molecular weight alcohol is ethanol.

65. The method according to claim 60, wherein step iii) comprises diluting a sub-sample of the solvent comprising the free fatty acids in a reaction mixture and performing a method according to any of claims 1 to 25.

66. The method according to claim 60, wherein step iii) comprises gas-chromatography, HPLC, or binding to a fluorescently modified fatty acid binding protein.